

ABSTRACT OF THE DISCLOSURE

A simple and accurate method for assay of a single-stranded RNA containing a specific nucleic acids sequence in a sample at almost constant temperature by using at least the following reagents (A) to (I), which comprises a step of adding the reagents (A) to (I) one by one (in any order), in combinations of at least two or all at once and a step of measuring a fluorescent signal in the presence of the reagent (I) at least once after addition of at least the reagents (A) to (H);

(A) a first single-stranded oligonucleic acid complementary to a sequence neighboring the 5' end of the specific nucleic acids sequence in the single-stranded RNA,

(B) a second single-stranded oligo DNA complementary to a 3'-end sequence within the specific nucleic acids sequence,

(C) an RNA-dependent DNA polymerase,

(D) deoxyribonucleoside triphosphates,

(E) a third single-stranded oligo DNA having (1) a promoter sequence for a DNA-dependent RNA polymerase, (2) an enhancer sequence for the promoter and (3) a 5'-end sequence within the specific nucleic acids sequence, in this order from the 5' end,

(F) a DNA-dependent DNA polymerase,

(G) a DNA-dependent RNA polymerase,

- (H) ribonucleoside triphosphates, and
 - (I) a fourth single-stranded oligo DNA complementary to the specific nucleic acids sequence which is labeled so that it gives off a measurable fluorescent signal on
- 5 hybridization with a nucleic acid containing the specific nucleic acids sequence.